

under reduced pressure to one half volume in order to remove most of the methanol. Water (600 mL) was added and the solution was heated to reflux, treated with charcoal (5 g) and hot filtered through Celite. The solution was allowed to cool to 25 °C. The resulting precipitate was collected, washed with water (200 mL) and dried at 90 °C/0.2 mmHg for 5 h to give a constant weight of 87.4 g (89%) of tan, crystalline solid; mp 247 °C (shrinks), 255 °C (dec, lit. (1) mp 250-252 °C); TLC homogenous (*R*_f 0.50, isopropanol-ammonium hydroxide-water 16:3:1); PMR (DMSO), δ 5.73 (d, 2, 2-NH₂), 5.78 (s, 1, H-1), 6.83 (br s, 2, 6-NH₂).

B. 2'-O-(2-methoxyethyl)-2,6-diaminopurine riboside and 3'-O-(2-methoxyethyl)-2,6-diaminopurine riboside

[0139] To a solution of 2,6-diaminopurine riboside (10.0 g, 0.035 mol) in dry dimethyl formamide (350 mL) at 0 °C under an argon atmosphere was added sodium hydride (60% in oil, 1.6 g, 0.04 mol). After 30 min., 2-methoxyethyl bromide (0.44 mol) was added in one portion and the reaction was stirred at 25 °C for 16 h. Methanol (10 mL) was added and the mixture was concentrated under reduced pressure to an oil (20 g). The crude product, containing a ratio of 4:1 of the 2'/3' isomers, was chromatographed on silica gel (500 g, chloroform-methanol 4:1). The appropriate fractions were combined and concentrated under reduced pressure to a semi-solid (12 g). This was triturated with methanol (50 mL) to give a white, hygroscopic solid. The solid was dried at 40 °C/0.2 mmHg for 6 h to give a pure 2' product and the pure 3' isomer, which were confirmed by NMR.

C. 3'-O-2-(methoxyethyl)guanosine

[0140] With rapid stirring, 3'-O-(2-methoxyethyl)-2,6-diaminopurine riboside (0.078 mol) was

dissolved in monobasic sodium phosphate buffer (0.1 M, 525 mL, pH 7.3-7.4) at 25 °C. Adenosine deaminase (Sigma type II, 1 unit/mg, 350 mg) was added and the reaction was stirred at 25 °C for 60 h. The mixture was cooled to 5 °C and filtered. The solid was washed with water (2x25 mL) and dried at 60 °C/0.2 mmHg for 5 h to give 10.7 g of first crop material. A second crop was obtained by concentrating the mother liquors under reduced pressure to 125 mL, cooling to 5 °C, collecting the solid, washing with cold water (2x20 mL) and drying as above to give 6.7 g of additional material for a total of 15.4 g (31 % from guanosine hydrate) of light tan solid; TLC purity 97%.

D. N²-Isobutyryl-3'-O-2-(methoxyethyl)guanosine

[0141] To a solution of 3'-O-2-(methoxyethyl)guanosine (18.1 g, 0.0613 mol) in pyridine (300 mL) was added trimethyl silyl chloride (50.4 mL, 0.46 mol). The reaction was stirred at 25 °C for 16 h. Isobutyryl chloride (33.2 mL, 0.316 mol) was added and the reaction was stirred for 4 h at 25 °C. The reaction was diluted with water (25 mL). After stirring for 30 min, ammonium hydroxide (concentrated, 45 mL) was added until pH 6 was reached. The mixture was stirred in a water bath for 30 min and then evaporated under reduced pressure to an oil. The oil was suspended in a mixture of ethyl acetate (600 mL) and water (100 mL) until a solution formed. The solution was allowed to stand for 17 h at 25 °C. The resulting precipitate was collected, washed with ethyl acetate (2x50 mL) and dried at 60 °C/0.2 mmHg for 5 h to give 16.1 g (85%) of tan solid; TLC purity 98%.

E. 5'-O-(DMT)-N²-isobutyryl-3'-O-(2-methoxyethyl) guanosine

[0142] A solution of N²-Isobutyryl-3'-O-2-(methoxyethyl) guanosine (0.051 mol) in pyridine (150 mL) was evaporated under reduced pressure to dryness. The residue was redissolved in pyridine

(300 mL) and cooled to 10-15 °C. DMT-Cl (27.2 g, 95%, 0.080 mol) was added and the reaction was stirred at 25 °C for 16 h. The reaction was evaporated under reduced pressure to an oil, dissolved in a minimum of methylene chloride and applied on a silica gel column (500 g). The product was eluted with a gradient of methylene chloride-triethylamine (99:1) to methylene chloride-methanol-triethylamine (99:1:1). Selected fractions were combined, concentrated under reduced pressure and dried at 40 °C/0.2 mmHg for 2 h to afford 15 g (15.5% from guanosine hydrate) of tan foam; TLC purity 98%.

EXAMPLE 8

[5'-O-(DMT)-N²-isobutyryl-3'-O-(2-methoxyethyl) guanosin-2'-O-(2-cyanoethyl-N,N-diisopropyl) phosphoramidite]

[0143] The protected nucleoside from Example 7 (0.0486 mol) was placed in a dry 1 L round bottom flask containing a Teflon stir-bar. The flask was purged with argon. Anhydrous methylene chloride (400 mL) was cannulated into the flask to dissolve the nucleoside. Previously vacuum dried N,N-diisopropylaminohydrotetrazolide (3.0 g, 0.0174 mol) was added under argon. With stirring, bis-N,N-diisopropyl-aminocyanoethylphosphoramidite (18.8 g, 0.0689 mol) was added via syringe over 1 min (no exotherm noted). The reaction was stirred under argon at 25 °C for 16 h. After verifying the completion of the reaction by TLC, the reaction was transferred to a separatory funnel (1 L). The reaction flask was rinsed with methylene chloride (2x50 mL). The combined organic layer was washed with saturated aq. sodium bicarbonate (200 mL) and then brine

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